ORIGINAL SUBMISSION

000001

03-03-28P03:49 RCVD

24 March 2003

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notice: Spirulina

Dear CFSAN,

Per proposed Section 21 CFR 170.36, Cyanotech Corporation (Kailua-Kona, HI) and Earthrise Nutritionals, Inc. (Petaluma, CA) hereby notify the Food and Drug Administration that they claim the use of Spirulina in certain food products is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act because the notifiers have determined that such use is Generally Recognized as Safe (GRAS). The basis for such determination is through scientific procedures. Cyanotech Corporation and Earthrise Nutritionals, Inc. equally accept responsibility for this GRAS determination.

Spirulina is a whole product of biological origin. It consists of the dried biomass of the cyanobacterium *Arthrospira platensis*. In this notice, we express our view, based on scientific procedures, that Spirulina is GRAS for use in foods such as bars, nutritional drink mixes and snacks, and as a condiment in salads and pasta, at levels ranging from 0.5 to 3 grams per serving. The present GRAS Notice expands upon our earlier submission (GRAS Notice No. GRN 000101), and clarifies the issues raised by the FDA regarding that earlier notice. In particular, we have clarified the subject of the Notice, its source and its means of identification. We ask the FDA to please incorporate by reference GRN 000101 and all materials therein into the present GRAS Notice for Spirulina. Additional supporting reference materials, not previously included in GRN 000101, accompany the present Notice.

Spirulina is not intended for use as a color additive. The intended use of Spirulina is similar to that of other approved GRAS substances such as Brown Algae (21 CFR 184.1120) and Red Algae (21 CFR 184.1121). Furthermore, we claim that Spirulina is not a color additive per 21 CFR 70.3 (f) as it is a food ingredient such as cherries, green or red peppers or chocolate that contributes its own natural color when mixed with other foods. The intended use of Spirulina is not similar to that of beet extract used to color lemonade a pink color.

000002

73-4460 Queen Kaahumanu Highway, #102 • Kailua-Kona, Hawaii 96740 • Phone (808) 326-1353 • Fax: (808) 329-3597

Spirulina has a long history and documented record of human consumption, and is known by prominent researchers to be safe and nutritious. In the past 20 years, it has been marketed and consumed as a safe human food by millions of people in North and South America, Asia, Europe, Australia and Africa. Furthermore, Spirulina has been approved as a food for human consumption by many governments, as well as health agencies and associations of over 70 countries. Based on 30 years of safety and quality research, many countries and organizations have established Spirulina quality and safety standards, which are abided to by Cyanotech Corporation and Earthrise Nutritionals, Inc. Spirulina is cultivated under scientifically controlled conditions that virtually eliminate contamination by other cyanobacteria and algae. Moreover, according to the US Food and Drug Administration, Spirulina can be legally marketed in the United States as a food as long as it is labeled accurately and contains no contaminated or adulterated substances (FDA Talk Paper #T81-18).

Numerous data published in the primary scientific literature, including human and animal safety studies and work with malnourished children, attest to the safety of dietary Spirulina. These data provide abundant evidence that there is a consensus among qualified experts, including the United Nations Food and Agriculture Organization (UNFAO) and United Nations Industrial Development Organization (UNIDO), that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

Thank you very much for your attention to our present GRAS Notice.

Sincerely,

John E. Dore, Ph.D.
Scientific Director, Cyanotech Corporation

Authorized Agent for Notifiers:

Cyanotech Corporation
73-4460 Queen Kaahumanu Hwy. #102
Kailua-Kona, HI 96740

Earthrise Nutritionals, Inc.
424 Payran St.
Petaluma, CA 94952

GRAS Notification: Spirulina

(1) Exemption Claim:

Per proposed Section 21 CFR 170.36, Cyanotech Corporation (Kailua-Kona, HI) and Earthrise Nutritionals, Inc. (Petaluma, CA) hereby notify the Food and Drug Administration that they claim the use of Spirulina in certain food products is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act because the notifiers have determined that such use is Generally Recognized as Safe (GRAS). The basis for such determination is through scientific procedures. Cyanotech Corporation and Earthrise Nutritionals, Inc. equally accept responsibility for this GRAS determination.

The intended use of Spirulina is similar to that of other approved GRAS substances such as Brown Algae (21 CFR 184.1120) and Red Algae (21 CFR 184.1121). Spirulina is not intended for use as a color additive. Furthermore, we claim that Spirulina is not a color additive per 21 CFR 70.3 (f) as it is a food ingredient such as cherries, green or red peppers or chocolate that contributes its own natural color when mixed with other foods. The intended use of Spirulina is not similar to that of beet extract used to color lemonade a pink color.

(i) Name and Address of Notifiers:

The notifiers for this GRAS determination are:

Cyanotech Corporation
74-4460 Queen Kaahumanu Hwy., #102
Kailua-Kona, HI 96740
Tel: 808-326-1353

Contact: John E. Dore, Ph.D.
Scientific Director
Email: jdore@cyanotech.com

Earthrise Nutritionals, Inc.
424 Payran Street
Petaluma, CA 94952
Tel: 760-348-5027 x21

Contact: Amha Belay, Ph.D.
Scientific Director
Email: **abelay@cts.com**

Dr. Dore is the authorized agent for this GRAS notification on behalf of both notifiers.

(ii) Common or Usual Name of the Notified Substance:

Spirulina is the common name of the notified substance. This substance is a whole product of biological origin, not a purified compound. The common name "Spirulina" refers to the dried biomass of the cyanobacterium *Arthrospira platensis*. Cyanotech Corporation holds trademarks to the names "Spirulina Pacifica" and "Hawaiian Energizer" for its Spirulina products, which are marketed as a human dietary supplements. Spirulina is also manufactured and marketed by Earthrise Nutritionals, Inc. as a dietary supplement under various trademarks including "Earthrise Spirulina", "Spirulina Bio" and "Spirulina Gold."

(iii) Applicable Conditions of Use:

Currently, Spirulina is manufactured into a powder, flakes or tablets and marketed as a dietary supplement. The intended GRAS use of the substance is as a source of protein and phytonutrients, such as carotenoids. Spirulina would be added to four general categories of food: (1) specialty food bars (e.g., granola, breakfast or energy bars), (2) powdered nutritional drink mixes such as "smoothies," (3) "healthy" snacks such as popcorn and (4) as a condiment for salads and pasta meals. The level of use for these foods would be from 0.5-3 grams of Spirulina per serving size. The population expected to consume these foods are those that are active, healthy and usually exercise on a regular basis. Vegetarians have long been typical and

traditional consumers of Spirulina. The substance is not intended for infant formulas. For each of the intended uses, Spirulina is not a color additive per 21 CFR 70.3 (f) as it is a food ingredient such as cherries, green or red peppers or chocolate that contributes its own natural color when mixed with other foods.

An estimated daily intake is calculated in the following manner:

High-end Consumer: We estimate that a high-end user may consume 2 servings of food per day containing the highest levels of Spirulina. This may be one powdered smoothie beverage and one food bar containing 3 grams of Spirulina each for a total of 6 grams of Spirulina per day.

Medium Consumer: A medium user may consume 1 serving of food per day containing the highest level of Spirulina. This may be one powdered smoothie or food bar containing 3 grams of Spirulina per day.

Low-end Consumer: A low-end user may consume 1-4 servings per month resulting in an exposure of 3-12 grams of Spirulina per month if at the highest levels.

Spirulina has been tested in a number of foods and beverages at these levels and does not have a negative impact on the organoleptic qualities (taste or smell) of these products.

(iv) Basis for GRAS Determination:

The basis for this GRAS determination of Spirulina is through scientific procedures. Cyanotech Corporation and Earthrise Nutritionals, Inc. equally accept responsibility for this GRAS determination.

(v) Data Availability:

Data and information that are the basis for the notifier's GRAS determination are available for FDA review and copying at reasonable times at Cyanotech Corporation (Kailua-Kona, HI) or Earthrise Nutritionals, Inc. (Petaluma, CA). Alternatively, the information will be sent to FDA upon request.

(2) Detailed Identity of the Notified Substance:

(i) Chemical Name:

Not applicable; the substance is a whole product of biological origin and is not a purified compound.

(ii) CAS Registry Number:

Not applicable; the substance is a whole product of biological origin and is not a purified compound.

(iii) Enzyme Commission Number:

Not applicable; the substance is a whole product of biological origin and is not a purified compound or enzyme preparation.

(iv) Empirical Formula:

Not applicable; the substance is a whole product of biological origin and is not a purified compound.

(v) Structural Formula:

Not applicable; the substance is a whole product of biological origin and is not a purified compound.

(vi) Quantitative Composition

(A) Physical Appearance of Spirulina

Spirulina is fine uniform powder, dark blue-green in color, with a mild seaweed taste and smell. It may also be formed into flakes or tablets.

(B) General Composition of Spirulina

Component	Percentage
Protein	53-62%
Carbohydrates	17-25%
Lipids	4-6%
Minerals	8-13%
Moisture	3-6%

(C) Typical Analysis of Minerals in Spirulina

Minerals	Amount (per 3 grams)
calcium	14 mg
magnesium	23 mg
iron	1.6 mg
phosphorus	30 mg
potassium	56 mg
sodium	42 mg
manganese	96 mcg
zinc	81 mcg
boron	90 mcg
copper	21 mcg
molybdenum	12 mcg
selenium	1.0 mcg

(D) Typical Analysis of Fatty Acids in Spirulina

Fatty Acids	Amount (per 3 grams)
Palmitic	61 mg
γ-linolenic (GLA)	28 mg
Linoleic	28 mg
Oleic	9.9 mg

Palmitoleic	4.2 mg
Stearic	2.5 mg
Eicosatrienoic	0.6 mg
Myristic	0.4 mg
Margaric	0.4 mg
Margaroleic	0.4 mg
Myristoleic	0.3 mg
Eicosadienoic	0.3 mg
Arachidonic	0.2 mg

(E) Typical Analysis of Vitamins in Spirulina

Vitamins	Amount (per 3 grams)
Vitamin B1 (thiamine HCl)	75 mcg
Vitamin B2 (riboflavin)	110 mcg
Vitamin B3 (niacin)	450 mcg
Vitamin B6	15 mcg
Vitamin B12 (human-active)	2.0 mcg

(F) Typical Analysis of Selected Phytonutrients in Spirulina

Phytonutrients	Amount (per 3 grams)
Cis-β-carotene	1.5 mg
Trans-β-carotene	5.3 mg
Zeaxanthin	3.0 mg
Chlorophyll a	24 mg
Phycocyanin (crude)	360 mg
C-Phycocyanin	165 mg

(vii) Method of Manufacture

(A) Source and Type of Cultivated Microorganism

Spirulina is a whole product of biological origin. It consists of the dried biomass of the cyanobacterium *Arthrospira platensis.* The source strain, cultivated by both Cyanotech Corporation and Earthrise Nutritionals, Inc., was obtained from the University of Texas at Austin Algae Culture Collection (UTEX). This strain, designated UTEX 1926, was originally isolated from an alkaline salt flat near Del Mar, California by R. Lewin in 1969. This production strain has undergone no genetic modification.

(B) Identification of Cultivated Microorganism

Arthrospira platensis is a filamentous cyanobacterium (oxygenic photosynthetic bacterium) with a worldwide distribution in fresh, marine, brackish and especially alkaline waters. The taxonomy of cyanobacteria (commonly referred to, erroneously, as blue-green "algae") as a group has recently undergone major revision due to the availability of modern genetic methods for determining microbial phylogeny. The most recent comprehensive treatise on the subject identifies *Arthrospira* as follows (Boone and Castenholz 2001):

Phylum BX. Cyanobacteria
Subsection III. (formerly Order Oscillatoriales)
Form-genus I. *Arthrospira*

It has been demonstrated using genetic techniques that the various strains of the form-genus *Arthrospira* are highly related, and probably are all representatives of a single nomen species (Scheldeman et al. 1999). For the purposes of this GRAS notice, we preserve the species designation *platensis* because the UTEX 1926 strain cultivated by both notifiers was originally classified under this designation. However, it should be understood that other *Arthrospira* "species" such as *A. fusiformis* and *A. maxima*, are

compositionally identical and are considered by taxonomists to be synonymous with *A. platensis*.

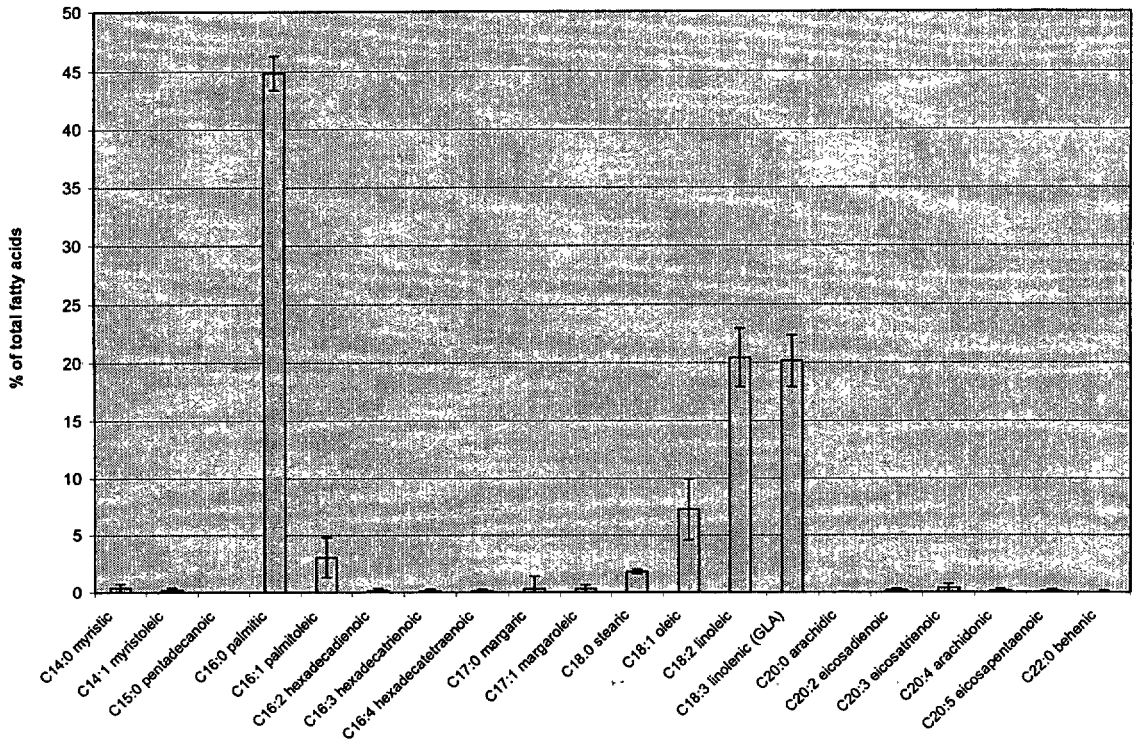
Arthrospira trichomes are typically 5-12 μm in diameter, and have a distinctive open helical morphology. Unfortunately, the helical morphology common with the otherwise distinct and smaller cyanobacterial form-genus *Spirulina* has led to much confusion regarding the proper designation of *Arthrospira* (Tomaselli et al. 1996). Over the years, the common name "Spirulina" has been applied to foodstuffs made from *Arthrospira* so pervasively that it would be a formidable task to attempt to retrain the consumers of Spirulina as to the correct taxonomic designation of the cultivated microorganism. Instead, we will retain the common name "Spirulina" to describe the dried product that is the subject of this notice, while making it clear that this dried product is derived from the cyanobacterium *Arthrospira platensis*.

Although it is usually possible to identify *Arthrospira platensis* microscopically, there are variations within the cultivated strain caused by environmental factors, such as the occasional "straightening" of the normal helical morphology (Lewin 1980). Positive and unambiguous identification can be obtained using either genetic or chemical "fingerprinting" techniques. Genetic techniques such as those used by Scheldeman et al. (1999) to determine the relatedness of *Arthrospira* strains are beyond the everyday capabilities of the notifiers. Instead, we employ chemical methods for ensuring that our *A. platensis* cultures are properly identified and substantially free of other cyanobacteria and algae. Substantial concentrations of phycocyanin and β-carotene within Spirulina are good indicators of genuine *Arthrospira*, but the most telling chemical identification is the unique fatty acid composition of *Arthrospira* (Romano et al. 2000; Ötleş and Pire 2001).

Genuine *Arthrospira* strains have a significant proportion of γ-linolenic acid (GLA), no α-linolenic acid (ALA), a low content of 16:1 fatty acids and a very low content of 16:2 fatty acids (Cohen and Vonshak 1991; Cohen et al. 1995). Cyanotech Corporation and Earthrise Nutritionals, Inc. have been monitoring the fatty acid profiles of their Spirulina products for nearly a decade; the results indicate constancy in the content of GLA and other fatty acids over time. From 1995 through 2001, Earthrise Spirulina

Spirulina maintained a GLA content of 1.15 ± 0.13 % of total weight, while from 1994 through 2001, Cyanotech Spirulina maintained its characteristic fatty acid profile with negligible variability (Fig. 1).

Figure 1. Fatty acids in Spirulina, 1994-2001, expressed as percent of total fatty acids.



(C) Composition of Growth Medium

Arthrospira platensis is cultivated in an alkaline aqueous medium rich in nutrient salts. The growth medium consists of water, sodium bicarbonate, nitrates, phosphates, sulfates, and trace minerals. The high pH and alkalinity of the growth medium inhibits the growth of potentially contaminating organisms, resulting in a virtual monoculture of *A. platensis*. Nutrients are supplied by reliable manufacturers that include specifications for heavy metals and other possible contaminants. No solvents, pesticides, herbicides or toxic substances are used during any cultivation or manufacturing step of the product. There are no carcinogens or compounds that are known in the scientific literature to

degrade or metabolize to carcinogens, used in the manufacturing process or known within Spirulina. Cyanotech Corporation and Earthrise Nutritionals, Inc. each have part of their Spirulina production dedicated to organic production and are certified by Quality Assurance International (San Diego, CA), under the National Organic Program of the U.S. Department of Agriculture.

(D) Manufacturing and Processing of Spirulina

Arthrospira platensis is grown in large, shallow mixed ponds that are lined with nylon scrim-reinforced polypropylene. This liner material is approved for potable water systems. Cultures are circulated in a closed circuit by means of a paddlewheel. This "raceway" design for outdoor mass cultivation of photosynthetic microorganisms was developed in the 1950s and is used widely in the industry (Dodd 1986). Nutrients are monitored and adjusted by laboratory chemists who conduct daily tests to assure consistency and optimal conditions. Ponds are harvested one to seven times per week. The culture is transferred with a pump through PVC pipes into a dedicated processing building, where it is passed over a series of stainless-steel screens to rinse and concentrate the biomass. The biomass slurry is then transferred by gravity to shaker screens for further concentration, and finally to a vacuum belt which accumulates the biomass as a paste and subjects it to a final washing step. The *Arthrospira platensis* paste is then pumped into a spray dryer to remove the moisture, resulting in the free flowing fine powder known commonly as Spirulina. The entire process from pond to powder takes less than 15 minutes.

Samples of the powder are collected in sterilized bags, labeled, and transferred to the Quality Control Laboratory for microbiological assays and other quality control assessments. The laboratory staff logs all data collected onto written sheets and into a database on the computer network; lots are graded to certain specifications according to customer requirements.

The dried powder is weighed and vacuum-sealed into various sized oxygen-barrier bags to minimize exposure to air and prevent possible oxidation of phytonutrients such as

β-carotene and fatty acids. The bags are then packed into cardboard boxes or drums, sealed with tape and labeled to reflect the package weight and lot numbers for tracking purposes. All reasonable precautions are taken to assure that production procedures do not contribute contamination such as filth, harmful chemicals, undesirable microorganisms, or any other objectionable material to the processed product.

(E) Facilities

Spirulina is presently cultivated and processed in two locations in the United States. The location of the facilities are:

Cyanotech Corporation
73-4460 Queen Kaahumanu Highway, #102
Kailua-Kona, HI 96740
Tel: 808-326-1353
FAX: 808-329-3597

Earthrise Nutritionals, Inc.
113 East Hoober Road (PO Box 270)
Calipatria, CA 92233
Tel: 760-348-5027
Fax: 760-348-2895

Cyanotech Corporation holds Food Establishment Permit issued by the State of Hawaii for Spirulina production and Earthrise Nutritionals, Inc. holds a registration with the State of California to manufacture, process, pack and hold food products.

(F) Process Controls

Spirulina powder is manufactured in accordance with Current Good Manufacturing Practices promulgated under the United States Federal Food, Drug and

Cosmetic Act and applicable Hawaii and California statutes and regulations. These laws assure that the facilities, methods, practices, and controls used in the manufacture, processing, packing, or holding of food products are in conformance with or are operated in conformity with Good Manufacturing Practices to assure that the food products are safe for consumption and have been prepared, packed, and held under sanitary conditions.

All operations in receiving, inspecting, transporting, packaging, segregating, preparing, processing, and storing of the product are conducted in accord with adequate sanitation principles. Raw materials and ingredients are inspected and segregated as necessary to assure that they are clean, wholesome, and fit for processing and are stored under conditions that will protect against contamination and minimize deterioration. Packaging materials do not transmit contaminants or objectionable substances to the product, and provide adequate protection from contamination.

The Quality Control Department and GMP Coordinator have the responsibility and authority to approve or reject all raw materials, in-process materials, packaging materials, final product and labeling, and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The Quality Control Department has the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the final product. Adequate laboratory facilities for the testing and approval (or rejection) of raw materials, in-process materials, packaging materials and final product are available to the Quality Control Department. The manufacturing facility is also audited and inspected for violations of Good Manufacturing Practices on a regular basis by the GMP Coordinator.

Cyanotech Corporation and Earthrise Nutritionals, Inc. have over 25 years of manufacturing experience with Spirulina sold into the health food market. Their facilities are subjected to periodic unannounced inspections from State and local regulatory agencies, and have never been cited for a major violation or issued a Form 483 violation of Good Manufacturing Practices. On October 7, 1996, Cyanotech Corporation was issued a Certificate of Registration from Orion Registrar Inc. for its ISO 9002-94 Quality

Management System (Certificate Identification HI-1-96-1 EAC/SIC Code: 13/2833). Earthrise Nutritionals, Inc. obtained ISO-9001 registration on July 31, 1998, from Perry Johnson Registrars, Inc. (Certificate Number 98-242). The ISO certificates are both currently in good standing. Earthrise Nutritionals, Inc. has also attained a NNFA Dietary Supplement Good Manufacturing Practices Certification as of May 2001.

Covance Laboratories, Inc. (Madison, WI) or Woodson-Tenent Laboratories, Inc. (Memphis, TN) periodically conduct tests on finished products, including proximate analyses, pesticide screens and assays for heavy metals.

(viii) Characteristic Properties

(A) General Properties

Spirulina is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying the biomass of the cyanobacterium, *Arthrospira platensis*. It consists of common proteins, carbohydrates, lipids, and minerals found in other plant products (Dillon et al. 1995). It is not readily soluble in water or solvents, but it forms a suspension when mixed into water.

(B) Spectrophotometric Properties

Phycocyanin, the major pigment in Spirulina, is water-soluble; when cells are ruptured and this pigment extracted into an aqueous solution, its characteristic blue spectrophotometric signature may be observed, with an absorption peak at a wavelength of 620 nm (Fig. 2). The major oil-soluble pigment in Spirulina is the carotenoid, β-carotene. When Spirulina is extracted with an organic solvent (acetone) and the pigments separated chromatographically and analyzed spectrophotometrically, the β-carotene fraction exhibits a characteristic absorption spectrum with twin peaks located at wavelengths of 450 nm and 476 nm (Fig. 3).

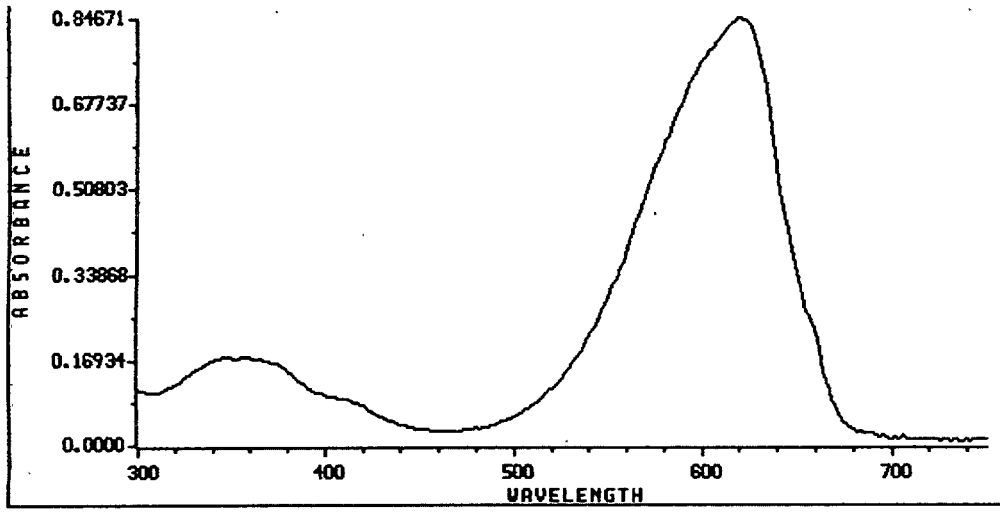
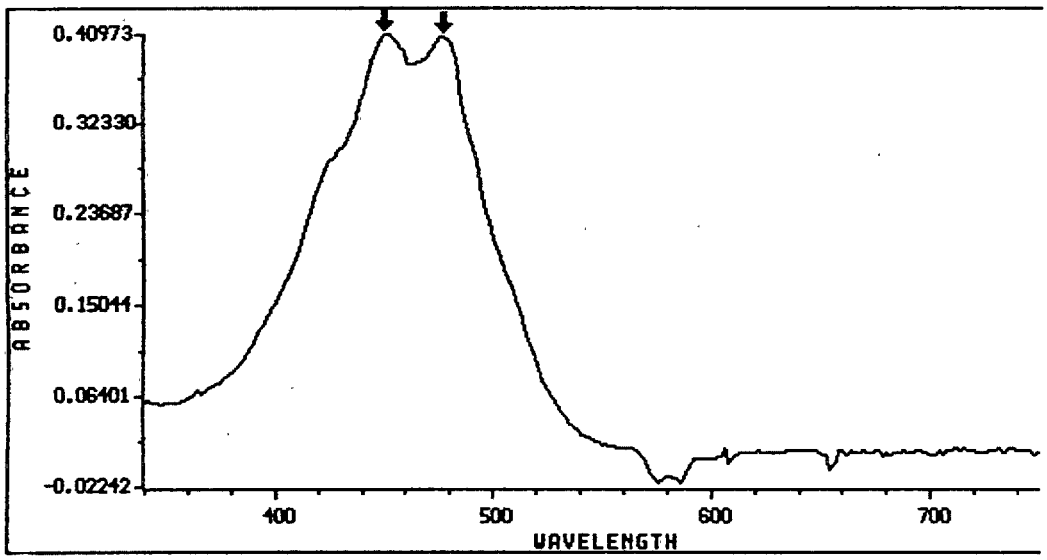


Figure 3. Absorbance spectrum of acetone-extracted β-carotene of Spirulina



(C) Assay for Substance

There is not a single assay for the substance as it is a whole product of biological origin and not a purified compound. However, certain components of the substance, such as carotenoids, phycocyanin and chlorophyll-a, can be assayed. The methods used by the

as carotenoids, phycocyanin and chlorophyll-a, can be assayed. The methods used by the two companies may vary slightly but generally follow the protocols below.

(1) HPLC Analysis of Spirulina Carotenoids and Spectrophotometric Analysis of Total Carotenoids

---

Part 1 Extraction in Methanol

Materials
Metric balance
Hach tubes (12 ml) with caps
Glass beads
Water bath (45 degree C.)
Vortexer
Centrifuge
Volumetric flask (25 ml)
DMSO
Methanol (Reagent Grade)
Graduated pipettes
Pasteur pipettes and rubber balls

Procedure
1) Perform the analysis in duplicate for each sample.
2) Weigh approximately 30 mg of Spirulina powder directly into Hach Tubes. Record the weight.
3) Add 3 grams of glass beads and 2.5 ml DMSO to each tube.
4) Tightly cap the tubes and vortex them briefly for 30 seconds.
5) Place the tubes into a 45 degree water bath for 20 minutes. Every 10 minutes remove tubes from the water bath and vortex them for 30 seconds.
6) After 20 minutes in the water bath, remove the tubes.
7) Add 5 ml of methanol to each tube, cap the tubes and vortex them vigorously for 30 seconds. Centrifuge the tubes at 4200 rpm for 3 minutes.
8) With a Pasteur pipette, pipette the supernatant from each tube into a volumetric flask.
9) Repeat step 7 and 8 until the centrifuged methanol is colorless. Three extractions should be ample.
10) After all the supernatant is collected in the volumetric flask, bring the flask up to volume with methanol.
11) Place the stopper in the volumetric flask and invert gently to mix the contents.

Part 2 Saponification and Preparation for HPLC

Materials
Methanol Extract obtained in Part 1
Saturated potassium chloride in water
25 % di-ethyl ether in hexane (reagent grade)
DI water
Hach tubes
vortexer
centrifuge
Sodium sulfate anhydrous (NaSO4)
Nitrogen manifold
Pasteur pipettes
3 ml volumetric pipette
1 ml calibrated pipette
3 ml volumetric flask
Running solvent (18 % acetone in hexane - HPLC grade)

Procedure
1) With the 3 ml volumetric pipette remove 3 ml of extract from the 25 ml volumetric flask and put it into a clean Hach tube.
2) Add 3 ml of 25 % diethyl ether in hexane to the tube.
3) Add 0.5 ml saturated KOH in water to the tube.
4) Cap the tube and vortex lightly to mix.
5) Place tube in a dark place for 30 minutes.
6) Remove the cap from the tube and add exactly 1 ml of DI water.
7) Cap the tube and vortex briefly to mix.
8) Centrifuge the tube at 4200 rpm for 3 minutes.
9) Add 1 gram of NaSO4- anhydrous to a clean tube - one for each sample.
10) Remove the ether-hexane layer from the centrifuge tube with a Pasteur pipette and put it into the Hach tube the NaSO4- anhydrous. Use additional aliquots (approximately 1 ml) of the 25 % ether in hexane to totally rinse out any color that remains in the top of the centrifuged tube where the solvent layer was. Combine all the solvent into the Hach tube with the NaSO4.
11) With the Pasteur pipette gently draw in and expel the solvent to expose the solvent to the NaSO4 to remove any water.
12) Transfer the solvent to a clean Hach tube, rinsing the NaSO4 with small aliquots of 25 % ether in hexane.
13) Evaporate the ether-hexane solvent from the Hach tube with a gentle stream of gas from the nitrogen manifold.
14) When the tube is completely dry add a small amount of running solvent to the tube and pipette the liquid into a 3 ml volumetric flask. Rinse the tube with the addition of small aliquots of running solvent and transfer it to the 3 ml volumetric flask. Bring the flask up

to volume.
15) Transfer the contents of the volumetric to a clean Hach tube for the HPLC analysis.

Part 3 HPLC Analysis

Materials
Luna column with Safety Guard pre-column
Solvent system, hexane:acetone (86:14)
Standard Operating Procedure for Beckman HPLC
Percent Dry Weight Calculation

Calculations:

Consult the SOP for the Beckman HPLC for detailed operating directions using method "sp2". After the run the calculation for sample amount is:

Sample Amount = (methanol (ml) / (sample wt (mg) X Percent dry wt (decimal))/10

E.g. Sample Amount =((25 ml) / (30 mg X .95))/10 = .0877192

Part 4 Spectrophotometric Analyses for Total Carotenoids

Materials
Methanol extract obtained in Part 1
2 ml pipette
3 ml volumetric pipette
15 ml conical glass centrifuge tubes, calibrated "to contain" with 0.1 ml graduations and screw top lids.
Saturated KOH in water
Diethyl ether
DI water
Vortex
Scanning Spectrophotometer
Pasteur pipettes
Dry weight of the Spirulina sample

Procedure
1) Analyze each sample in duplicate.
2) Pipette exactly 2 ml of the methanol extract in to a 15 ml centrifuge tube.
3) Add exactly 3 ml of diethyl ether with a volumetric pipette to the Hach tube.
4) Add 0.33 of saturated KOH to the tube for a short saponification. Cap the tube and vortex briefly to mix. Set the samples in a dark place for 15 – 30 minutes for saponification.

5) Remove the cap and add 5 ml of water to each sample.
6) Replace the cap and vortex the tube vigorously for 30 seconds. Centrifuge the tubes for 3 minutes at 4200 rpm.
7) The ether layer should contain all the yellow pigments and the aqueous layer should be a pale blue-green.
8) Remove the cap from the centrifuge tube and add diethyl ether to the ether layer until it is exactly at a 3 ml volume according to the increments on the tube.
9) The absorbance of the ether layer is read on the spectrophotometer. Read the maximum absorbance (approximately 450-453) against a diethyl ether blank.

Calculation

$$\text{Total Carotenoids (percent)} = \frac{\text{Max Abs (450-453)}}{259.2 \times \text{sample wt (mg)} \times \text{dry wt}} \times 25 \text{ ml} \times 1.5 \times 100$$

(2) Quantitative Analysis of C-Phycocyanin from Spirulina

Background:

Spirulina and other blue-green algae contain c-phycocyanin, which acts as an accessory pigment when light energy is captured and transferred to chlorophyll a. This is a spectrophotometric method adapted to extract and quantify a relatively pure c-phycocyanin fraction from Spirulina.

Equipment and instruments:

Spectrophotometer at 620nm
Refrigerator (4 C)
Phosphate buffer (pH 7.0)*
10 ml centrifuge tubes
Cooled centrifuge (10 C @ 3500 RPM)
Desiccator
Weigh pans
Analytical balance

Dry Weight

1) Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.
2) When pans are cool, weigh and record weight of pan.
3) Tare the balance with the pan on it and place about two grams of powder in the pan.
4) Record the weight of the powder.

5) Place pan and powder in the oven to dry for six hours.
6) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
7) Weigh and record the total weight of the pan and the dry powder.
8) Perform duplicates for each sample.

Dry Weight Calculations

$$\text{Percent dry wt} = \frac{(\text{pan (g)} + \text{dried powder (g)}) - \text{pan wt (g)}}{\text{powder wt (not dried) (g)}}$$

Phycocyanin Assay

1) Perform analysis in duplicate.
2) Weigh accurately 30 mg. Spirulina powder into a 10-ml centrifuge tube.
3) Add 10 mls. of the 100 mM phosphate buffer (100-mM Phosphate buffer contains 10.64 g. K_2HPO_4 and 5.29g. KH_2PO_4 per liter, pH 7.).
4) Vortex to mix well.
5) Store in refrigerator overnight.
6) Vortex to mix well.
7) Centrifuge 5 minutes at 10 C at 3500 RPM.
8) Read absorbency of each replicate at 620 nm, using phosphate buffer as blank.
9) Average absorbency readings for dilution replicates.

Derivation of pure C-Phycocyanin:

$$\%\ \text{pure CPC} = \frac{A_{620} \times (10) \times (100)}{7.3 \times (\text{mg. sample}). \times (\%\ \text{dry wt.})}$$

where 7.3 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

Derivation of crude C-Phycocyanin:

$$\%\ \text{crude CPC} = \frac{A_{620} \times (10) \times (100)}{3.39 \times (\text{mg. sample}). \times (\%\ \text{dry wt.})}$$

where 3.39 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

Reference:

Boussiba S. and A. Richmond. 1979. Isolation and Purification of Phycocyanins from the Blue-Green Alga *Spirulina platensis*. Arch. Microbiol. 120:155-159.

(3) Quantitative Analysis of Chlorophyll-a from Spirulina

Background:

Spirulina contains only Chlorophyll-a. Carotenoids and chlorophyll are acetone soluble but the carotenoids do not interfere in this spectrophotometric method as the absorbency for carotenoids range from 400-500 nm, and the absorbency for chlorophyll A is 666 nm.

The other major pigments in *Spirulina* are the water-soluble phycocyanin. These remain in an acetone-insoluble pellet during the assay.

Equipment and instruments:

Analytical balance
Centrifuge
Drying pans
Drying oven at 110 degrees C.
85 % acetone in water
Spectrophotometer
35 ml round bottom glass centrifuge tubes with caps
50 ml volumetric flask with lid
Vortexer (Maxi Mix II)
Centrifuge
Glass beads (through 20 mesh)
Pipettes

Dry Weight

1) Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.
2) When pans are cool, weigh and record weight of pan.
3) Tare the balance with the pan on it and place about two grams of powder in the pan. Record the weight of the powder.
4) Place pan and powder in the oven and dry for two hours.
5) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
6) Weigh and record the total weight of the pan and the dry powder.
7) Perform duplicates for each sample.

Chlorophyll A Assay

1) Weigh approximately 50 mg of Spirulina into a 35 ml centrifuge tube. Record weight.
2) Add 5 grams of glass beads and 2.5 ml of 85 % acetone in water.
3) Vortex vigorously for 5 minutes.
4) Add 10 ml of 85% acetone in water, vortex briefly and centrifuge at 3200 RPM of 5

minutes.
5) Collect the supernatant in a 50 ml volumetric flask .
6) Repeat steps 3-5 until supernatant is clear. Four extractions should be sufficient.
7) Bring the flask up to volume with 85% acetone in water and cap the flask and invert gently to mix the contents.
8) Read the absorbency with the spectrophotometer at 666nm and 642 nm against an 85 % acetone/water blank.

Calculations:

Dry weight

$$\text{Percent dry wt} = \frac{\text{(pan (g)+ dried powder (g)) - pan wt (g)}}{\text{powder wt (not dried) (g)}}$$

Chlorophyll a

$$\text{Chlorophyll a (\%)} = \frac{[(9.93 \times Abs_{666}) - (0.0777 \times Abs_{642})] \times 0.05 \text{ liter} \times 100}{\text{Sample weight (mg)} \times \text{\% dry wt.}}$$

References

A.O.A.C. Official Methods of Analysis (1995); 940.03

(ix) Content of Potential Human Toxicants

Spirulina is produced without the use of solvents, pesticides, herbicides, antibiotics or hormones. The *Arthrospira platensis* cultures grown for Spirulina production at Cyanotech Corporation and Earthrise Nutritionals, Inc. have never been genetically modified or engineered. Spirulina is not subjected to irradiation. There are no known carcinogens or compounds that may be degraded or metabolized to carcinogens used in the manufacturing process or known to occur naturally within Spirulina. Periodic screening of Spirulina for potentially harmful substances is conducted by Covance Laboratories, Inc. (Madison, WI) or Woodson-Tenent Laboratories, Inc. (Memphis, TN). These screenings include assays for heavy metals and pesticides, and support our specifications for food-grade material below.

(x) Specifications for Food-Grade Material

Both Cyanotech Corporation and Earthrise Nutritionals, Inc. adhere to the quality

specifications for food-grade Spirulina presented below. In some cases, the specifications for a particular constituent or contaminant may actually be stricter for one or the other company than the minimum specifications presented.

(A) General Specifications

Protein	>52 %
Minerals	<14 %
Moisture	<7 %
β-carotene	>120 mg/100g
Total carotenoids	>380 mg/100g
C-phycocyanin	>5 %

(B) Contaminant Specifications

Arsenic	<1.0 ppm
Cadmium	<0.5 ppm
Lead	<1.0 ppm
Mercury	<0.05 ppm
Pesticides	negative
Rodent hairs	<10/10g
Insect fragments	<50/10g

(C) Microbiological Specifications

Total aerobic bacteria	<200,000 cfu/g
Total coliforms	<10 cfu/g
E. coli	negative
Salmonella	negative
Staphylococcus aureus	negative

(D) Product Stability

Shelf life	3 years

Carotenoids of Spirulina are the most sensitive component and can degrade due to heat, light and oxygen. Precautions are taken during manufacturing and processing to limit these conditions as much as possible. Dried Spirulina is packed into airtight bags or bottles and then vacuum-sealed before shipping. Labeling of the substance includes pertinent information about shelf life and the best storage and handling conditions for optimal stability. A stability study was conducted by Earthrise Nutritionals, Inc. on Spirulina stored in airtight bags over a duration of five years:

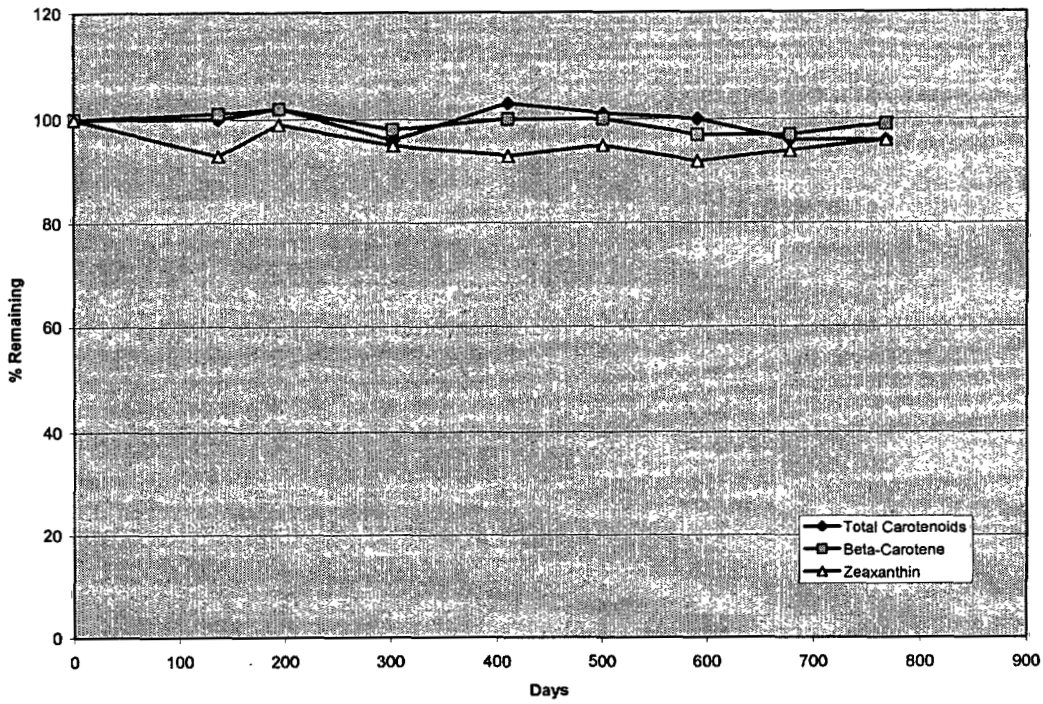
	Specification	Initial	1yr	2 yr	3 yr	4 yr	5 yr
Appearance:	dark blue-green	same	same	same	same	same	same
Consistency:	powder	same	same	same	same	same	same
Smell:	mild seaweed smell	same	same	same	same	same	same
Taste:	mild seaweed taste	same	same	same	same	same	same
Phycocyanin:	8.6%	8.6%	8.6%	8.2%	8.0%	7.5%	7.0%
Carotenoids:	450 mg/100g	450	450	425	400	350	300
E. coli.:	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Salmonella:	neg.	neg.	neg.	neg.	neg.	neg.	neg.

Another stability study was conducted by Cyanotech Corporation on Spirulina packaged in sealed glass bottles (Fig. 4).

(3) Self-limiting Levels:

There are no self-limiting levels for Spirulina. The substance is currently marketed as a dietary supplement in the form of powder, tablets or flakes. The taste and smell of the substance is similar to mild seaweed.

Figure 4. Stability of carotenoids in Spirulina packaged in sealed glass bottles.



(4) Detailed Summary of GRAS Determination:

We claim the use of Spirulina in certain food products is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act because the we have determined that such use is GRAS. The basis for such determination is through scientific procedures.

(i) Summary of GRAS Determination Through Scientific Procedures:

(A) Discussion of Safety Data

(1) Use of Spirulina as a Foodstuff

Spirulina has an ancient history as a human food. It was regularly consumed by the Aztecs in North America prior to the Spanish conquest in the 1500s (Ciferri

Tutalcingo lakes with fine-meshed nets and dried the paste under the sun. The harvested biomass was made into cakes 3-4 centimeters thick called "techuitlatl." These cakes were made into breads and were regularly traded as a commodity.

Spirulina has also been consumed for centuries as a major source of protein by the Kanembu people who live along the shores of Lake Chad in Africa. *Arthrospira* is collected from the water's edge in fine-woven baskets, transferred to clay pots or gourds, and dried under the sun into small biscuits called "dihé." Dihé is combined into the majority of sauces and is eaten in up to 70% of their meals, amounting to about 10-12 grams per person per meal. In times of famine, dihé is a main ingredient of their diets (Delpeuch et al. 1976; Ciferri 1983; Abdulqader et al. 2000).

Packaged Spirulina has been marketed and consumed as a human food for over 20 years and has been approved as a food for human consumption by governments, health agencies and associations representing over 70 countries:

Argentina	Australia	Austria	Bahrain	Bahamas	Bangladesh
Belarus	Belgium	Brazil	Bulgaria	Canada	Chad
Chile	China	Colombia	Costa Rica	Croatia	Czech Republic
Denmark	Ecuador	Egypt	Ethiopia	Finland	France
Germany	Greece	Guam	Gulf States	Haiti	Hong Kong
Hungary	India	Iceland	Indonesia	Ireland	Israel
Italy	Jamaica	Japan	Kenya	Korea	Kuwait
Liechtenstein	Luxembourg	Macedonia	Malaysia	Mexico	Myanmar
Monaco	Netherlands	New Zealand	Nigeria	Norway	Peru
Philippines	Poland	Portugal	Romania	Russia	Saudi Arabia
Singapore	Slovenia	South Africa	Spain	Sweden	Switzerland
Taiwan	Thailand	Togo	Turkey	Ukraine	United Kingdom
United States	Venezuela	Vietnam	Yugoslavia	Zaire	Zimbabwe

Spirulina is commercially produced in numerous countries including the United States, China, India, Thailand, Taiwan, and Japan. The annual production is estimated to be over 3000 metric tons. Currently, Cyanotech Corporation cultivates *Arthrospira platensis* in 57 ponds, each with an average size of 2,900 square meters. The annual production capacity is approximately 400,000 kg of Spirulina per year. It is estimated that Cyanotech has produced a total of 3,500,000 kg of Spirulina for human consumption since operations began in 1985. Earthrise Nutritionals, Inc. cultivates *Arthrospira platensis* in 30 ponds with an average size of 5,000 square meters each. The facility has an annual production capacity of 500,000 kg and has also produced some 3,500,000 kg since its operation began in 1982. Currently, it is estimated that 250,000 kg of Spirulina is imported from China and India into the U.S. for human consumption each year. The following Spirulina production estimates (in metric tons) are based on a world survey of researchers and producers (Henrikson 1989):

Year	Burma	Chile	China	Cuba	India	Japan	Mexico	Taiwan	Thailand	Vietnam	USA-CA	USA-HI	Total
1975	0	0	0	0	0	0	20	0	0	0	0	0	20
1976	0	0	0	0	0	5	45	0	0	0	0	0	50
1977	0	0	0	0	0	11	65	4	0	0	0	0	80
1978	0	0	0	0	0	20	145	4	1	0	0	0	170
1979	0	0	0	0	0	20	200	9	50	0	1	0	280
1980	0	0	0	0	0	20	245	14	50	0	1	0	330
1981	0	0	0	0	0	30	250	19	50	0	1	0	350
1982	0	0	0	0	0	35	250	25	60	0	20	0	390
1983	0	0	0	0	0	45	250	25	60	0	50	0	430
1984	0	0	1	0	0	47	250	60	75	0	55	2	490
1985	0	0	1	0	0	53	250	60	100	1	55	10	530
1986	0	0	1	0	1	60	250	60	110	3	55	20	560
1987	0	0	3	0	3	60	250	60	110	4	70	40	600
1988	0	0	3	0	3	60	250	80	110	4	70	50	630
1989	0	0	8	0	6	50	250	80	110	6	70	60	640
1990	0	0	8	0	7	35	250	90	120	0	120	80	710
1991	4	1	8	0	7	20	250	90	120	0	160	100	760
1992	12	4	12	0	12	20	250	90	120	0	160	120	800
1993	15	5	20	2	20	20	225	90	120	3	160	120	800
1994	20	5	60	10	80	20	100	80	130	5	210	160	870
1995	25	7	120	20	150	20	0	60	150	8	370	250	1170
1996	30	20	250	40	250	20	0	60	150	10	480	400	1710
1997	30	50	500	50	250	20	0	70	150	20	500	450	2090
1998	30	70	700	60	300	20	100	80	150	20	600	500	2630
1999	30	90	900	70	500	20	100	80	150	20	800	600	3360

In the 1960s and 1970s, interest in Spirulina as a foodstuff centered primarily on its value as a rich source of protein. More recently, Spirulina has been used widely as a dietary supplement because it is a good source of vitamin A (as β-carotene) and other dietary carotenoids normally found in fruits and vegetables. It has been recommended by experts to eat 5 servings of fruits and vegetables per day, yielding a typical daily intake of 20-60 mg of β-carotene (33,000-100,000 IU of vitamin A activity), 10-30 mg of α-carotene, and 3-6 mg each of lutein, lycopene and zeaxanthin (Yarnell 1999). Spirulina is a good source of β-carotene (0.2%) and zeaxanthin (0.1%), and contains smaller amounts of other natural carotenoids.

(2) Animal Safety Studies

In the 1970s and early 1980s, Spirulina underwent extensive safety studies with animals. Independent feeding tests in France, Mexico, Italy, Japan and India showed no undesirable results and no toxic side effects on rats and pigs, even when Spirulina constituted a significant portion of the dietary protein (Février and Sève 1976; Krishnakumari et al. 1981; Ciferri 1983; Jassby 1988). Long-term (18-month) feeding studies with rats similarly revealed no toxicity or adverse effects (Boudène et al. 1976). In 1980, one of the most comprehensive Spirulina animal studies was sponsored by the United Nations Industrial Development Organization (UNIDO) using rats and mice. In this study, Spirulina was incorporated into the food at 10-35% of the total diet. There were no problems with second or third generation reproduction, fertility, lactation or birth defects found. No cancer causing properties were found. No significant problems with heavy metals, nucleic acids, pesticides or bacteria were found (Chamorro 1980).

Toxicology research continued through the 1980s and 1990s, showing Spirulina has no peri- or postnatal toxicity in rats, creates no adverse effects on reproduction (including male and female fertility), does not affect duration of gestation or the number of abnormal offspring, and displays no negative effects on the uterus or ovaries (Becker and Venkataraman 1984; Chamorro et al. 1988, 1997; Salazar et al. 1996, 1998). In a separate study, Spirulina was incorporated into experimental diets at levels of 10-30% and fed to groups of adult CD-1 mice. Five day short-term and prolonged-term (5 d/week for 10 weeks) feeding was followed by mating with untreated virgin females. Examination of uteri and ovaries of pregnant females on day 12-14 of gestation for counting preimplantation losses and non-living implants failed to reveal dominant lethal effects (Chamorro and Salazar 1989). In a study conducted in India, pregnant rats were fed five different diets providing 22% protein to study the supplementary effects of Spirulina. Rats receiving Spirulina produced significantly

higher litter sizes than those with casein and wheat gluten. Birth weights of the pups from the Spirulina group were comparable to the control group. The authors concluded that Spirulina appears to be a good dietary supplement during pregnancy (Kapoor and Mehta 1993).

A chronic intoxication test with Spirulina was conducted in Japan on Wistar rats of both sexes for six months. Rats were fed ad libitum either a 20% Spirulina diet or a control. The weight, appearance, and growth and histology of organs (brain, heart, stomach, liver, spleen, kidneys, testes or ovaries and adrenal glands) were not significantly different between the experimental and control groups at the end of the study period. Hematological tests showed some statistical differences (e.g. Hb and SGPT of the male and total bilirubin of the female), though abnormal findings were not detected. The authors concluded that the Spirulina diet did not cause any toxic effects over the 6 months of feeding (Yoshino et al. 1980).

Two experiments with weaned pigs were conducted to evaluate the feasibility of partially replacing soy protein in a basal corn-soybean meal with proteins from Spirulina. There were no signs of diarrhea, loss of appetite, toxicity, or of gross histopathological lesions of the gastrointestinal tract, kidney, liver, or femur in pigs fed any of the diets. Blood hemoglobin and serum protein, albumin and urea concentrations were similar among all groups in the experiments. It was suggested that at least one-half of the protein supplied by soybean meal can be replaced in the diet of the early weaned pig by Spirulina without adverse effects (Yap et al. 1982). In another study of Spirulina's potential as a foodstuff, young rats reared for 15 weeks on a diet of 5% Spirulina grew favorably with good food digestion efficiency and protein levels. The amount of fat accumulated was small and fat synthesis from carbohydrates was similar to that of the control group. A reduction in the amount of fat in the liver was observed (Watanabe and Takai 1986).

Kidney toxicity caused by mercury was suppressed by feeding a water-soluble extract of Spirulina to rats. Renal toxicity in rats caused by p-aminophenol and cisplatin was also significantly reduced by this phycocyanin-rich extract of Spirulina.

Researchers concluded that Spirulina might be applicable to the reduction of general renal dysfunction (Fukino et al. 1990).

(3) Human Safety Studies

In 1970, the attention of the United Nations Food and Agriculture Organization (UNFAO) was attracted by the fact that humans in Africa were consuming blue-green "algae". The UNFAO organized an educational campaign in Chad to encourage consumption of Spirulina harvested from natural sources of *Arthrospira platensis*. More than 6000 meals were distributed under the supervision of the UNFAO and the campaign was considered a success (Institut Francais du Petrol 1970). Another report stated "dihé" (Spirulina sauce) was served at the school canteen where the majority were the Kanembu people. The consumption of Spirulina in the children's food caused no problems of illness. Spirulina was also consumed by non-Kanembu people at Fort Lamy, now called Ndjemena (Fadoul 1971).

Spirulina was given to malnourished children and adults in clinical studies beginning in the early 1970s. Since the late 1970s, millions of people in developed countries have used it as a health food supplement, taking 3 to 20 grams a day. Rarely are there any reports of allergies or sensitivities.

A one year feeding program in India with 5,000 pre-school children showed that a Spiulina-supplemented diet reduced the occurrence of "Bitot's spot", a symptom of vitamin A deficiency, from 80% to 10%. These rural children near Madras consumed 1 gram of Spirulina per day for at least 150 days. This small amount provided the daily requirement of vitamin A (as β-carotene), which can help prevent blindness and eye diseases (Seshadri 1993). In another study with 400 school children, a daily dose of β-carotene from Spirulina increased their vitamin A status to the same level as those administered pure vitamin A. Spirulina was given to children in extruded noodles, sweetened with sugar to preserve the beta-carotene. Called "Spiru-

Om", it was well accepted by the children. The project was sponsored by the Indian Government (Seshadri 1993).

In Romania, Spirulina tablets were given to 21 patients with various nutritional deficiencies. They had suffered weight loss in conjunction with gastric resection, tubercular infection, chronic pancreatitis and gastritis, rheumatoid arthritis, anemia and diabetes mellitus. With Spirulina, the patients gained weight and their protein profiles improved (Fica et al. 1984). At Nanjing Children's Hospital in China, 27 children, 2-6 years old, recovered in a short period from bad appetite, night sweats, diarrhea and constipation when consuming a baby nourishing formula containing 1.5g of Spirulina, 12g baked barley sprout, vitaimn B1 and zinc. The clinical effects showed Spirulina was safe and healthy for children (Ren 1987).

High zinc Spirulina may be twice as effective as a zinc supplement in curing zinc deficiency in children. In trials conducted in Jiangxi, China, the effective dose of zinc from Spirulina was 2 to 4 times less than the zinc from a common supplement, zinc sulfate. One hundred children diagnosed as suffering from zinc deficiency were tested for a three-month period. Fifty children were given zinc sulfate and 50 were given Spirulina tablets. Doctors concluded that Spirulina's effect was superior to that of zinc sulfate. Spirulina had no side effects and was easy to administer for long periods of time. They theorized that high zinc Spirulina had many bioactive and nutritious substances, which improved mineral absorption, general health and the immune system (Yonghuang et al. 1994).

Spirulina has about a 4% content of nucleic acids (DNA and RNA), lower than *Chlorella* and other microalgae, yeast and fungi (6-11%). One study found that uric acid levels did not increase in humans taking up to 30 grams a day of *Chlorella* protein or 50 grams of *Chlorella* (Waslien et al. 1970). Since Spirulina is lower in nucleic acid content, eating up to 50 grams a day can be safely used as major protein source (Jassby 1988).

Thirty healthy men with high cholesterol, mild hypertension and hyperlipidemia showed lower serum cholesterol, triglyceride and LDL levels after eating Spirulina for eight weeks. These men did not change their diet, except for adding Spirulina. No adverse effects were noted. Group A consumed 4.2 grams of Spirulina daily for eight weeks. Total serum cholesterol dropped a significant 4.5% within 4 weeks from 244 to 233. Group B consumed Spirulina for four weeks, then stopped. Serum cholesterol levels decreased, then returned to the initial level. The researchers concluded that dietary Spirulina did lower serum cholesterol and was likely to have a favorable effect on alleviating heart disease since the arteriosclerosis index improved (Nakaya et al. 1988).

The bioavailability of total carotenes and β-carotene from Spirulina was examined in apparently healthy preschool children in India and found to be comparable to those values reported for other plant sources like leafy vegetables and carrots. This study also showed Spirulina is a good source of vitamin A, as there was a significant increase in serum retinol levels. Researchers concluded that Spirulina can be used as a source of vitamin A in the diet, is relatively inexpensive, has higher β-carotene than most other plant sources and can be cultivated throughout the year (Annapurna et al. 1991).

The chemopreventive potential of Spirulina (1 g/day for 12 months) was evaluated in reversing oral leukoplakia in pan tobacco chewers in Kerala, India. Complete regression of lesions was observed in 20 of 44 subjects supplemented with Spirulina, as opposed to 3 of 43 in the placebo group. When considered by the type of leukoplakia, the response was more pronounced in homogeneous lesions: complete regression was seen in 16 of 28 subjects with homogeneous leukoplakia, 2 of 8 with erythroplakia, 2 of 4 with verrucous leukoplakia and 0 of 4 with ulcerated and nodular lesions. Within one year of discontinuing supplements, 9 of 20 responding subjects from the Spirulina group developed recurrent lesions. Supplementation with Spirulina

did not increase serum concentrations of retinal or beta-carotene, nor was it associated with toxicity (Mathew et al. 1995).

Spirulina reduced urine radioactivity levels by 50% in only 20 days among children at the Institute of Radiation Medicine in Minsk, Belarus. This result was achieved with a dose level of 5 grams of Spirulina per day. The Institute has developed a program to treat 100 children every 20 days. This 1993 report confirms 1990-91 research on the beneficial health effects of Spirulina on children with radiation sickness (Loseva and Dardynskaya 1993). It concludes: "Use of Spirulina decreases radioactive dose load received from food contaminated with radionuclides, Cesium-137 and Strontium-90. Spirulina is favorable for normalizing the adaptative potential of children's bodies in conditions of long-lived low dose radiation.".

A critical review of data on the safety and therapeutic effects of Spirulina, which range from reduction of cholesterol and cancer to enhancing the immune system, increasing intestinal lactobacilli, reducing nephrotoxicity by heavy metals and drugs, and radiation protection, was published in 1993 (Belay et al. 1993). More recently, a number of scientific publications have presented new data supporting the role of dietary Spirulina in human health. These include evidence of stimulation of secretion of cytokines from blood mononuclear cells (Mao et al. 2000), augmentation of interferon production (Hirahashi et al. 2002), inhibition of HIV-1 replication (Ayehunie et al. 1998) and reversal of age-induced decreases in cerebellar β-adrenergic function (Gemma et al. 2002). A comprehensive review of the science behind the application of Spirulina as a nutitional and therapeutic supplement has also recently been published (Belay 2002).

(4) Heavy Metals

Cyanotech Corporation and Earthrise Nutritionals, Inc. have published strict standards for heavy metals in Spirulina. Annual testing of composite samples for heavy metals reveals extremely low and often undetectable levels of lead, arsenic,

cadmium and mercury. The standard for mercury was set at less than 0.05 parts per million (ppm). In comparison, the US FDA standard in 'aquatic animals' is 1.0 ppm, permitting over 20 times more mercury. Standards were also set for cadmium (less than 0.05 ppm), lead (less than 1.0 ppm), and arsenic (less than 1.0 ppm). By comparison, the UN Protein Advisory Group standard for single cell protein permits higher heavy metals: 1.0 ppm for mercury; 1.0 ppm for cadmium, 5.0 ppm for lead; and 2.0 ppm for arsenic.

(5) Cyanobacterial Toxins

An important quality control issue surrounding production of cyanobacteria is the possibility of inadvertently harvesting other cyanobacteria containing cyanotoxins. This is a risk when harvesting algae from natural bodies of water containing mixed populations of phytoplankton, but is unlikely to be a problem with the tightly controlled *Arthrospira platensis* monocultures utilized by the notifiers. Nevertheless, because certain cyanobacterial and algal toxins are capable of causing widespread poisoning of animals and humans (Carmichael 1994), the notifiers take the issue very seriously.

In 1995-96, a group of leading microalgae and cyanobacteria producers including Cyanotech Corporation and Earthrise Nutritionals, Inc. sponsored research conducted by phytoplankton toxicologists. The result was a Technical Booklet for the Microalgae Biomass Industry as a guide to the use of a very sensitive enzyme linked immunosorbant assay (ELISA) and a protein phosphate inhibition assay (PPIA) for the detection of toxic microcystins and nodularins. These methods can detect, monitor and control cyanotoxins, so producers can assure a safe, nutritious product for human and animal food supplements (An and Carmichael 1996).

Spirulina is periodically assayed for microcystin and nodularin toxins by ELISA analysis using in-house testing as well as independent testing at Wright State University, Dayton, OH. Cyanotech Corporation and Earthrise Nutritionals, Inc. have

never had any detectable amount of microcystin or nodularin toxins in their Spirulina products.

(6) Other Approvals of Spirulina as Safety Evidence

According to the US Food and Drug Administration, Spirulina can be legally marketed in the United States as a food as long as it is labeled accurately and contains no contaminated or adulterated substances (FDA 1981).

(B) Information that Appears to be Inconsistent for GRAS Determination

The Special Nutritionals Adverse Event Monitoring System (http://vm.cfsan.fda.gov/~dms/aemsfull.html) lists approximately 50 incidents in which Spirulina was involved in an adverse event. However, in most of these cases, Spirulina was a minor component and other ingredients such as ma-huang, guarana, or gotu kola could be attributed to causing the symptoms. A few of the cases are also likely misidentified as Spirulina and were actually derived from a wild fresh-water cyanobacterium harvested from Klamath Lake called *Aphanizomenon flos-aquae*. This cyanobacterium is usually marketed as "Super Blue-Green Algae" and products made from it are often confused with Spirulina. *Aphanizomenon flos-aquae* has been associated with microcystin toxins in recent years.

In 1986, two papers published by Drs. Phyllis Johnson and L. Elliot Shubert reported high mercury levels (9.5 ppm) in commercial Spirulina (Johnson and Shubert 1986a,b). In a correspondence with Dr. E. W. Becker (Johnson 1986), Dr. Johnson admitted that her measurements suffered from an iron interference and were probably erroneous. Regrettably, it appears that Dr. Johnson never formally retracted her published data. Unsafe mercury levels in Spirulina have never been reported by any other researchers or independent testing laboratories.

(C) Basis for Consensus on Safety

Spirulina has a long history and documented record of human consumption, and is known by prominent researchers to be safe and nutritious. In the past 20 years, it has been marketed and consumed as a safe human food by millions of people in North and South America, Asia, Europe, Australia and Africa. Furthermore, Spirulina has been approved as a food for human consumption by many governments, as well as health agencies and associations of over 70 countries. Based on 30 years of safety and quality research, many countries and organizations have established Spirulina quality and safety standards, which are abided to by Cyanotech Corporation and Earthrise Nutritionals, Inc. Spirulina is cultivated under scientifically controlled conditions that virtually eliminate contamination by other cyanobacteria and algae. Moreover, according to the US Food and Drug Administration, Spirulina can be legally marketed in the United States as a food as long as it is labeled accurately and contains no contaminated or adulterated substances (FDA 1981).

Numerous data published in the primary scientific literature, including human and animal safety studies and work with malnourished children, attest to the safety of dietary Spirulina. These data provide abundant evidence that there is a consensus among qualified experts, including the United Nations Food and Agriculture Organization (UNFAO) and United Nations Industrial Development Organization (UNIDO), that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

References:

Abdulqader, G., L. Barsanti, and M. R. Tredici. 2000. Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. J Appl. Phycol. 12: 493-498.

An, J., and W. W. Carmichael. 1996. Technical booklet for the microalgae biomass industry: Detection of microcystins and nodularins using an enzyme linked immunosorbant assay (ELISA) and a protein phosphatase inhibition assay (PPIA). Dept. of Biological Sciences, Wayne State University, Dayton, OH.

Annapurna, V., N. Shah, P. Bhaskaram, M. Bamji, and V. Reddy. 1991. Bioavailability of *Spirulina* carotenes in preschool children. J. Clin. Biochem. Nutr. 10: 145-151.

Ayehunie, S., A. Belay, T. W. Baba, and R. M. Ruprecht. 1998. Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). J. Acquired Immune Deficiency Syndrome Human Retrovirol. 18: 7-12.

Becker, W. E., and L. V. Venkataraman. 1984. Production and utilization of the blue-green alga *Spirulina* in India. Biomass 4: 105-125.

Belay, A. 2002. The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. J. Am. Nutraceutical Assoc. 5(2): 27-48.

Belay, A., Y. Ota, K. Miyakawa, and H. Shimamatsu. 1993. Current knowledge on potential health benefits of *Spirulina*. J. Appl. Phycol. 5: 235-241.

Boone, D. R., and R. W. Castenholz [eds.]. 2001. The *Archaea* and the deeply branching and phototrophic *Bacteria*. Bergey's manual of systematic bacteriology, 2nd ed., vol. 1. Springer Verlag, New York.

Boudène, C., E. Collas, and C. Jenkins. 1976. Recherche et dosage de divers toxiques minereaux dans les algues spirulines de différentes origins, et évaluation de la toxicité a long terme chez le rat d'un lot algues spirulines de provenance Mexicaine. Ann. Nutr. Alim. 30: 577-588.

Carmichael, W. W. 1994. The toxins of cyanobacteria. Sci. Am., Jan. 1994, pp. 78-86.

Chamorro, G. 1980. Toxicological studies on *Spirulina* alga: Sosa Texcoco S.A. pilot plant for the production of protein from *Spirulina* alga. United Nations Industrial Development Organization, UF/MEX/78/048.

Chamorro, G. A., G. Herrera, M. Salazar, S. Salazar, and V. Ulloa. 1988. Subchronic toxicity study in rats fed *Spirulina*. J. Pharm. Belg. 43: 29-36.

Chamorro G., S. Salazar, L. Favila-Castillo, C. Steele, and M. Salazar. 1997. Reproductive and peri- and postnatal evaluation of *Spirulina maxima* in mice. J. Appl. Phycol. 9: 107-112.

Chamorro G. A., and M. Salazar. 1989. Dominant lethal assay of *Spirulina maxima* in male CD-1 mice after short-term and prolonged-term feeding. J. Food Protection 52: 125-127.

Ciferri, O. 1983. *Spirulina*, the edible microorganism. Microbiol. Rev. 47: 551-578.

Cohen, Z., M. C. Margheri, and L. Tomaselli. 1995. Chemotaxonomy of cyanobacteria. Phytochem. 40: 1155-1158.

Cohen, Z., and A. Vonshak. 1991. Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. Phytochem. 30: 205-206.

Delpeuch, F., A. Joseph, and C. Cavelier. 1976. Consommation alimentaire et apport nutritionnel des algues bleues (*Oscillatoria platensis*) chez quelques populations du Kanem (Tchad). Ann. Nutr. Alim. 29: 497-516.

Dillon, J. C., A. P. Phuc, and J. P. Dubacq. 1995. Nutritional value of the alga *Spirulina*. World Rev. Nutr. Diet. 77: 32-46.

Dodd, J. C. 1986. Elements of pond design and construction. In: Richmond, A. [ed.], CRC handbook of microalgal mass culture. CRC Press, Boca Raton, Florida, pp. 265-283.

Fadoul, L. 1971. Les algues bleues du Kanem. Rapport de mission par L. Fadoul (director du CNTA), A. Avrem et G. Le Guedes (experts de la division de la nutrition, FAO) Juin 1971.

Food and Agriculture Organization of the United Nations.

FDA. 1981. *Spirulina*. Talk Paper #T81-18. U.S. Food and Drug Administration.

Février, C. and Sève, B. Essais d'incorporation de spiruline (Spirulina maxima) dans les aliments des porcins. Ann. Nutr. Alim. 29: 625-650.

Fica, V., D. Olteanu, and S. Oprescu. 1984. Observations on the utilization of *Spirulina* as an adjuvant nutritive factor in treating some diseases accompanied by a nutritional deficiency: preliminary study. Rev. Med. Interna 36: 225-232.

Fukino, H., Y. Takagi, and Y. Yamane. 1990. Effect of *Spirulina* (*S. platensis*) on the renal toxicity induced by inorganic mercury and cisplatin. Proceedings of the 15th symposium on environmental pollutants and toxicology, November 9-10, 1989, Sendai, Japan. Eisei Kagaku, 36: P-5.

Gemma, C., M. H. Mesches, B. Sepesi, K. Choo, D. B. Holmes, and P. C. Bickford. 2002. Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar β-adrenergic function and increases in proinflammatory cytokines. J. Neurosci. 22: 6114-6120.

Hirahashi, T., M. Matsumoto, K. Hazeki, Y. Saeki, M. Ui, and T. Seya. 2002. Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. Int. Immunopharmacol. 2: 423-434.

Institut Francais du Petrol. 1970. Etat d'avancement du procede IFP de production d'algues. Rapport ou Comite Consultatif des Proteines, OAA/ OMS/ FISE, Dec. 1970, p.11.

Jassby, A. 1988. *Spirulina*: a model for microalgae as human food. In: Lembi, C. A., and J. R. Waaland [eds.], Algae and human affairs. Cambridge University Press, Cambridge, pp. 149-179.

Johnson, P.E. 1986. Letter to Dr. E. W. Becker, September 4, 1986. Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota.

Johnson, P. E., and L. E. Shubert. 1986a. Availability of iron to rats from *Spirulina*, a blue-green alga. Nutr. Res. 6: 85-94.

Johnson, P. E., and L. E. Shubert. 1986b. Accumulation of mercury and other elements by *Spirulina* (Cyanophyceae). Nutr. Rep. Int. 34: 1063-1070.

Kapoor, R., and U. Mehta. 1993. Effect of supplementation of blue green alga (Spirulina) on outcome of pregnancy in rats. Plant Foods Human Nutr. 43: 29-35.

Krishnakumari M. K., H. P. Ramesh, and L. V. Venkataraman. 1981. Food safety evaluation: Acute oral and dermal effects of the algae *Scenedesmus acutus* and *Spirulina platensis* on albino rats. J. Food Protect. 44: 934-935.

Lewin, R. A. 1980. Uncoiled variants of *Spirulina platensis* (Cyanophyceae: Oscillatoriaceae). Arch. Hydrobiol. 60 (suppl.): 48-52.

Loseva, L. P., and I. V. Dardynskaya. 1993. *Spirulina*- natural sorbent of radionuclides. 6th Int'l Congress on Applied Algology, September 6-11, 1993, Trebon, Czech Republic.

Mao, T. K., J. Van de Water, and M. E. Gershwin. 2000. Effect of Spirulina on the secretion of cytokines from peripheral blood mononuclear cells. J. Medicinal Food 3: 135-140.

Mathew, B., R. Sankaranarayanan, P. P. Nair, C. Varghese, T. Somanathan, B. P. Amma, N. S. Amma, and M. K. Nair. 1995. Evaluation of chemoprevention of oral cancer with *Spirulina fusiformis*. Nutr. Cancer 24: 197-202.

Nakaya, N., Y. Homma, and Y. Goto. 1988. Cholesterol lowering effect of *Spirulina*. Nutr. Rep. Int. 37: 1329-1337.

Ötleş, S., and R. Pire. 2001. Fatty acid composition of Chlorella and Spirulina microalgae species. J. AOAC Int. 84: 1708-1714.

Ren, M.J. 1987. *Spirulina* in Jiangxi China. Society of Applied Algology meeting, September 1987, Lille, France.

Romano, I., M. R. Bellitti, B. Nicolaus, L. Lama, M. C. Manca, E. Pagnotta, and A. Gambacorta. 2000. Lipid profile: a useful chemotaxonomic marker for classification of a new cyanobacterium in *Spirulina* genus. Phytochem. 54: 289-294.

Salazar, M., G. A. Chamorro, S. Salazar, and C. E. Steele. 1996. Effect of *Spirulina maxima* consumption on reproduction and peri- and postnal development in rats. Food Chem. Toxicity 34: 353-359.

Salazar, M., E. Martínez, E. Madrigal, L. E. Ruiz, and G. A. Chamorro. 1998. Subchronic toxicity study in mice fed *Spirulina maxima*. J. Ethnopharmacol. 62: 235-241.

Scheldeman, P., D. Baurain, R. Bouhy, M. Scott, M. Mühling, B. A. Whitton, A. Belay, and A. Wilmotte. 1999. *Arthrospira* ('*Spirulina*') strains from four continents are resolved into only two clusters, based on amplified ribosomal DNA restriction analysis of the internally transcribed spacer. FEMS Microbiol. Lett. 172: 213-222.

Seshadri, C. V. 1993. Large scale nutritional supplementation with *Spirulina* alga. All India Coordinated Project on Spirulina. Shri Amm Murugappa Chettiar Research Center (MCRC) Madras, India.

Tomaselli, L., M. R. Palandri, and M. R. Tredici. 1996. On the correct use of the *Spirulina* designation. Algological Stud. 83: 539-548.

Waslien, C., D. H. Calloway, S. Margen, and F. Costa. 1970. Uric acid levels in men fed algae and yeast as protein sources. J. Food Sci. 35: 294-298.

Watanabe, T., and Y. Takai. 1986. Effect of Spirulina (*Spirulina platensis*) on growth of rat. Hygiene Junior Univ. Bull. 4: 29-34.

Yap, T. N., J. F. Wu, W. G. Pond, and L. Krook. 1982. Feasibility of feeding *Spirulina maxima*, *Arthrospira platensis* or *Chlorella sp.* to pigs weaned to a dry diet at 4 to 8 days of age. Nutr. Rep. Int. 25: 543-552.

Yarnell, E. 1999. Carotenoids. Continuing Education Module, New Hope Institute of

Retailing, in association with Bastyr University, Bothell, Washington.

Yonghuang, W., et al. 1994. The study on curative effect of zinc containing *Spirulina* for zinc deficient children. 5th International Phycological Congress, June 1994, Qingdao, China.

Yoshino, Y., Y. Hirai, H. Takahashi, N. Yamamoto, and N. Yamazaki. 1980. The chronic intoxication test on *Spirulina* product fed to Wistar-strain rats. Jap. J. Nutr. 38: 221-225.

Pages 000046 - 000051 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000052 - 000074 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 0000075 - 000099 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000100 - 000103 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000104 - 000105 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000106 - 000124 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000125 - 000133 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000134 - 000145 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

United States Department of Agriculture
Agricultural Research Service
Northern States Area
Grand Forks Human Nutrition Research Center
2420 2nd Avenue, N
P.O. Box 7166
University Station
Grand Forks, North Dakota
58202-7166

September 4, 1986

Dr. W. Becker
Med. Univ. Klinik, Abt.II
Immunpathol. Labor
7400 Tübingen 1
Federal Republic of Germany

Dear Dr. Becker:

Thank you for your thoughtful letter about our paper on iron and Spirulina.

Your comments on the mercury analysis come at a time when we are reevaluating those data. We have data from ICAP analysis showing 10-20ppm Hg in ten different brands of Spirulina. Since our Hg standards gave us acceptable results and tests for matrix problems showed none, we had thought them correct. However, we have acquired a second ICAP instrument with better spectral resolution, and it turns out that the wavelength originally used for our Hg analysis is overlapped by an Fe line. The high levels of Fe in the samples could cause false Hg results. Indeed, a re-analysis on the new instrument under conditions where Fe did not interfere with the Hg determination, showed nondetectable levels of Hg (<1ppb). We are still puzzled why our original analysis of certified reference materials turned out OK and our tests for interference of Fe with the Hg showed no problem, so we are conducting a third analysis by a potentiometric method which is completely independent. I don't have those results yet. However, I think that our high Hg values will be confirmed to be in error.

The material used in the Nutr.Res. article originated in the USA. We had also analyzed other brands of commercial Spirulina with origins around the world. However we took the labels at face value and did not check identity of the material.

You are probably right about problems caused by rinsing the cultured algae with distilled water. I was not involved with the algal culture, as Dr. Shubert, my co-author, is experienced in algal culture. However, I should have thought of that problem. With regard to the test meal feeding, it is common practice in this type of study to offer the test meal for only about 4 hr. The animals were fasted overnight before the meal, so they were hungry, and most ate it in less than 4 hr. The meals were weighed out and any food remaining after the test meal was also weighed. Because the test meal was only 2.5g. of food, it was completely eaten by most rats (within 0.03g.). Rats which ate significantly less than the rest of the group were excluded from statistical analysis. Animals had free access to water during the test meal period.

I hope that I've answered your questions. If you want more information, I'll be glad to provide whatever I can.

Sincerely,

000146

Pages 000147 - 000152 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 0000153 - 000157 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000158 - 000162 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000163 - 000168 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000169 - 000175 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000176 - 000181 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000182 - 000191 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Reference List for Industry Submission, GRN 000127

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
0000075 - 000099	Boone, David R.; Castenholz, Richard W.; Garrity, George M.	The Archaea and the Deeply Branching and Phototrophic Bacteria	2001	Bergeys Manual of Systematic Bacteriology	Volume 1, pgs 474-543
0000153 - 000157	Johnson, Phyllis E.; Shubert, L. Elliot	Accumulation Of Mercury And Other Elements By Spirulina Cyanophyceae	December 1986	Nutrition Reports International	Volume 34, Number 6
000046 - 000051	Ayehunie, Seyoum; Belay, Amha; Baba, Timothy W.; Ruprecht, Ruth M.	Inhibition of HIV-1 Replication by an Aqueous Extract of Spirulina platensis (Arthrospira platensis)	1998	Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology	Volume 18, pgs 7-12
000052 - 000074	Belay, Amha	The Potential Application of Spirulina (Arthrospira) as a Nutritional and Therapeutic Supplement in Health Management	2002	The Journal of the American Nutraceutical Association	Volume 5, Number 2, pgs 27 - 48
000100 - 000103	Cohen, Zvi; Margheri, Maria Cristina; Tomasel, Luisa	Chemotaxonomy of Cyanobacteria	1995	Phytochemistry	Volume 40, Number 4, pgs 1155
000104 - 000105	Cohen, Zvi; Vonshak, Avigad	Fatty Acid Composition of Spirulina and Spirulina-Like Cyanobacteria In Relation To Their Chemotaxonomy	1991	Phytochemistry	Volume 30, Number 1, pgs 205-206
000106 - 000126	Dodd, Joseph	Elements of Pond Design and Construction	NA	Handbook of Microalgal Mass Culture	pgs 265 - 283
000127 - 000133	Richmond, Amos		1986	CRC Handbook of Microalgal Mass Culture	
000134 - 000145	Hirahashi, Tomohiro; Matsumoto, Misako; Hazeki, Kaoru; Saeki, Yoshiko; Ui, Michio; Seya Tsukasa	Activation of the human innate immune- system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of Spirulina platensis	2002	International Immunopharmacology	Volume 2, pgs 423-434
000147 - 000152	Johnson, Phyllis E.; Shubert, Elliot	Availability of Iron to Rats From Spirulina, A Blue-Green Alga	1986	Nurtrition Research	Volume 6, pgs 85-94

NA- Not applicable

Pages	Author	Title	Publish Date	Publisher	BIB_Info
000158 - 000162	Lewin, Ralph A.	Uncoiled variants of Spirulina platensis (Cyanophyceae: Oscillatoriaceae)	1980	Algological Studies	Supplement 60, pgs 48-52
000163 - 000168	Mao, T.K.; Water, Van De; Gershwin, M.E.	Effect of Spirulina on the Secretion of Cytokines from Peripheral Blood Mononuclear Cells	2000	Journal of Medicinal Food	Volume 3, Number 3, pgs 135 -140
000169 - 000175	Otles, Semih; Pire, Ruhsen	Fatty Acid Composition of Chlorella and Spirulina Microalgae Species	2001	Journal of AOAC International	Volume 84, Number 6, pgs
000176 - 000181	Romano, Ida; Bellitti M. Rosaria; Nicolaus, Barbara; Lama, Licia; Manca, M. Cristina; Pagnotta, Eduardo; Gambacorta, Agata	Lipid profile: a useful chemotaxonomic marker for classification of a new cyanobacterium in Spirulina genus	2000	Phytochemistry	Volume 54, pgs 289-294
000182 - 000191	Tomaselli, Buisa, Palandri, Maria Rosa; Tredici, Mario R.;	On the correct use of Spirulina designation	1996	Algological Studies	Volume 83, pgs 539-548

NA- Not applicable